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A role for ATP in tubular fibrosis in the diabetic kidney

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Aims: Recent studies link elevated hemi-channel mediated ATP release to increased tissue fibrosis in disease. This study examines a role for ATP in extracellular matrix (ECM) remodelling and increased secretion of fibrotic mediators in kidney tubular epithelial cells.

Methods: Human kidney (HK2) tubular epithelial cells were treated with ATPγS (1-100μM) for 48hrs. Cell morphology and cytoskeletal reorganisation were assessed by phase contrast microscopy and immunocytochemistry respectively. Cell viability was determined by MTT and crystal violet assays. Collagen I, IV, Fibronectin and Laminin expression were determined by immunoblot analysis, whilst Interleukin-6 and Beta-Nerve Growth Factor (β-NGF) secretion were assessed by cytokine arrays.

Results: ATPγs (1-100μM) failed to evoke any significant change in HK2 morphology, cytoskeletal reorganisation or cell viability at 48hrs. Immunoblotting confirmed that ATPγs up-regulated Collagen I to 177±12%, 182±21%, and 187±21% (n=3 $P<0.01$) and Collagen IV to 233±26%, 344±18.5%, and 390±10% compared to control (n=3 $P<0.001$) at 1, 10 and 100 μM. In addition, ATPγs significantly increased expression of Fibronectin to 274±47%, 350±23%, and 433±81% of control (n=3 $P<0.01$) at 1, 10 and 100 μM, yet failed to alter expression of ECM protein Laminin. Array analysis of supernatant from ATPγs treated cells confirmed a significant increase in secretion of both IL-6 and β-NGF to 261±24 and 194±44 respectively compared to control (n=3 $P<0.01$).

Conclusions: The current study suggests that glucose-evoked increases in hemi-channel mediated ATP release may contribute to the underlying pathology of tubulointerstitial fibrosis in the diabetic kidney.

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